Blanching Improves Anthocyanin Absorption from Highbush Blueberry (*Vaccinium corymbosum* L.) Purée in Healthy Human Volunteers: A Pilot Study

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ABSTRACT: Blueberries (*Vaccinium corymbosum* L.) are rich sources of phenolics and anthocyanins (ACNs). We investigated the absorption of ACNs after consumption of one portion (300 g) of minimally processed blueberry purée (P) obtained from blanched (BL) or unblanched (NB) berries. A repeated-measures, crossover design study was conducted on healthy human volunteers. Blood was drawn between baseline and 24 h after BL-P or NB-P consumption, while urine were collected from the day before the experiment up to 48 h. Total plasma ACN content was not significantly different, while phenolics content was higher in BL-P with respect to NB-P. The maximum ACN absorption in plasma was observed after 1.5 h from the intake of the purées and was significantly higher ($p \leq 0.05$) after the intake of BL-P. Both products increased the excretion of hippuric acid in urine. In conclusion, blanching had no significant effect on total ACN content and enhanced their absorption from minimally processed purées.

KEYWORDS: Blueberry, blanching, anthocyanins, absorption, human

■ INTRODUCTION

Berry fruits (e.g., blueberries, blackberries, bilberries, blackcurrants, cranberries, and strawberries) are rich sources of polyphenol bioactive compounds, such as phenolic acids, flavanols, and flavonoids, including anthocyanins (ACNs). ACNs comprise the largest group of natural, water-soluble pigments that provide the bright colors to flowers and berry fruits. They are generally concentrated in the skins of fruits, and their content is usually proportional to color intensity. Blueberries contain different ACNs, such as cyanidin, peonidin, petunidin, malvidin, and delphinidin. These compounds possess significant antioxidant capacity and play a key role in the prevention of oxidative stress by scavenging reactive oxygen species and free radicals, as demonstrated in various *in vitro* and *in vivo* studies.[1−3] Moreover, ACNs play a potential beneficial role in human health by reducing risks of several diseases, such as cardiovascular diseases and cancer.[4−6] The health benefit is strictly dependent upon their absorption, metabolism, distribution, and excretion. Studies focusing on ACN absorption have shown that their bioavailability is lower than that of other flavonoids and less than 1% of consumed amounts of ACNs (180−215 mg/day) is generally absorbed.[7] Their absorption occurs from the stomach and small intestine by different mechanisms that may involve a specific enzyme, such as bilitraslocase.[8] ACNs enter in the circulatory system after passing through the liver, move in the blood, and are distributed to different tissues. They appear in plasma within 15−60 min following consumption, and their maximum concentration is in the order of nanomolar levels.[7,9] Mostly, ACNs reach the colon, where they are extensively metabolized by intestinal microbiota, which contribute to the bioavailability and bioefficacy of ACNs and phenolic compounds in the systemic circulation.[10] Moreover, we have recently documented that ACNs and phenolic compounds can positively modulate the composition of intestinal microbiota and promote the colonization of the gastrointestinal tract by beneficial bacteria (i.e., *Bifidobacterium* spp. and *Lactobacillus* spp.) at the expense of unhealthy bacteria.[10,11]

Particular attention must be paid to the chemical changes that ACNs undergo during processing, so that they may be able to exert and maintain their health benefits. Because the availability of blueberries is seasonal, processing for long-term storage is desirable by the food industry. The majority of berries, including blueberries, are consumed as processed foods: juices, purées, jams, syrups, jellies, and various ready-to-drink beverages. The influence of processing on the content of food polyphenols and ACNs has been previously studied.[12−20] Significant losses of phenolic compounds compared to fresh fruits have been observed in blueberry juices,[13−15] raspberry purée and jam,[16] strawberry jams,[17,18] canned fruit,[18] and nectar.[19] Hager et al., demonstrated that processing of berries canned in water or syrup reduced total ACNs by about 42 and 51%, respectively.[20] Patras et al. observed that ACNs from blackberry and strawberry purée were reduced by about 28% after a thermal treatment of 20 min.[12] ACN degradation in

processed berry products results mainly from oxidation reactions generated by polyphenol oxidase and peroxidase enzymes and direct heat damage.[21,22] Other factors, such as pH, storage temperature, light, oxygen, proteins, and metallic ions, influence ACN stability and could negatively affect their bioavailability. [23]

Some studies have reported that the inclusion of a blanching preconditioning step, applied to inactivate oxidative enzymes before tissue mechanical processing, may improve ACN recovery in berry products.[14,21,24,25] Whole fruit thermal processing may influence not only phenolic abundance but also the food matrix environment and microstructure, with possible implications on the bioaccessibility of phenolic compounds.[26−28] Insight into the variables linking thermal treatments to phenolics recovery and bioavailability would provide information for not only researchers but also the food industry, which is interested in the development of innovative healthy products to place in the market. The low absorption of dietary ACNs is widely recognized, but the influence of food processing on the ACN absorption rate has been poorly studied.

The aim of this study was to investigate whether blanching whole berries could affect the absorption of ACNs from two blueberry purées in healthy human volunteers.

■ MATERIALS AND METHODS

Chemicals. Standards of cyanidin (Cy)-, delphinidin (Dp)-, peonidin (Pn)-, petunidin (Pt)-, and malvidin (Mv)-3-*O*-glucoside (glc), -3-*O*-galactoside (gal), and Cy- and Mv-3-*O*-arabinoside (Ara) were purchased from Polyphenols Laboratory (Sandnes, Norway). The Folin−Ciocalteau reagent and standards of gallic acid, chlorogenic acid, and cyanidin-3,5-diglucoside (CydG), as the ACN internal standard (IS), were purchased from Sigma-Aldrich (St. Louis, MO). Folin−Ciocalteau was from Fluka Biochemicals. Sodium carbonate, sodium acetate, metaphosphoric acid (MPA), potassium chloride, potassium metabisulfite, hydrochloric acid, methanol, acetonitrile, acetone, formic acid, and trifluoroacetic acid (TFA) were from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q apparatus (Millipore, Milford, MA).

Plant Material. A total of 30 kg of blueberry fruit (*Vaccinium corymbosum* L., cv. Brigitta) were cleaned, individually quick frozen (IQF) in a tunnel (Thermolab, Codogno, Italy) operating at −50 °C air temperature and 4.5 m/s air speed, divided into three lots (L1, L2, and L3) of 10 kg each, and stored at −20 °C for 1 month until analysis (L1) and processing (L2 and L3). Each lot of berries was divided into three aliquots (3.3 kg/aliquot) and processed into three trials (one trial/day). L2 was processed into purée (P) after partial thawing at 20 °C for 1 h (NB), while L3 was immediately processed after steam-blanching for 3 min and tap water-cooling in a pilot steam-blanching tunnel (Ghizzoni Dante and Figlio, Felino, Parma, Italia).[12] Purées were prepared by homogenizing berries for 1 min in a commercial food processor (Moulinex, Paris, France). Then, 300 g aliquots of purées were packed in 400 mL plastic vessels, sealed under partial vacuum using a 25 μm thick polypropylene film, and frozen within 20 min at −20 °C. From each lot of processed berries, 30 purée servings were obtained, randomly divided into two groups, and assigned to biochemical analysis (10 servings) and absorption trials (20 servings).

Subject Recruitment. A total of 10 healthy male subjects, aged 20.8 ± 1.6 years with a body mass index (BMI) of 22.5 ± 2.1 kg/m², were recruited from the student population of the University of Milan according to the following inclusion criteria: no smoking, no history of cardiovascular, diabetes, hepatic, renal, or gastrointestinal diseases, and not having taken any supplement, drug, or medication for at least 1 month before the beginning of the study. Subjects were selected on the basis of an interview to evaluate their dietary habits and ensure that they were as homogeneous as possible, in particular for fruit and vegetable consumption. This was obtained by means of a food

frequency questionnaire previously published and specifically revised to focus on food sources rich in antioxidants.[29] Exclusion criteria were high (>5 portions/day) or low (<2 portions/day) intake of fruit and vegetables and habitual alcohol consumption (<3 drinks per week were tolerated). Volunteers who followed a specific diet (e.g., vegetarian, vegan, or macrobiotic) and those who had a specific aversion for blueberry consumption were excluded. The inclusion criteria were sex (male), ethnicity (western European), BMI within normal range (from 19 to 25 kg/m²), age (from 19 to 30 years), physical activity (more than 30 min/day and from 2 to 4 days per week), and dietary habits as homogeneous as possible. On the basis of the questionnaire, 10 volunteers were selected. All participants gave informed consent, and the study was approved by the Ethics Committee of the University of Milan.

Experimental Design. Subjects were deprived of ACN-rich food sources 10 days before experimentation. Each volunteer received a complete list of foods to be avoided; the list included ACN-rich foods, such as berry fruits (i.e., blueberries, cranberries, raspberries, blackcurrants, and elderberries) red wine and red/violet fruits (i.e., grapes, cherries, pomegranates, red apples, plums, eggplants, tomatoes, and peppers), and other colored products (i.e., marmalade, jams, and juices containing berries). The repeated measure crossover design was chosen to limit interindividual variability. Subjects were randomly divided into two groups of five subjects each and consumed a portion of blanched purée (BL-P) or unblanched purée (NB-P) in the following order: group 1 was assigned to the sequence BL-P/wash-out/NB-P, whereas group 2 followed the sequence NB-P/wash-out/BL-P. The two experimental treatments were separated by a 10 day wash-out period. Breakfast, lunch, and dinner were standardized 1 day before the experiment and up to 48 days after purées consumption. Breakfast consisted of milk and biscuits (i.e., shortbread), while lunch consisted of two sandwiches (one with cooked ham and cheese and one with raw ham). For dinner, subjects could eat as a first dish (pasta or rice with butter and cheese) and a steak with potatoes and two slices of white bread. Coffee, tea, wine, beer, and chocolate were not allowed. Moreover, subjects were asked to exclude all ACN-containing foods as previously reported. On the scheduled day, after an overnight fast, subjects consumed a single dose of thawed NB-P or BL-P, providing approximately 300 mg of ACNs. The purées were consumed early in the morning, and blood was collected by a phlebotomist at time 0 (before the consumption of blueberry purées) and after 1, 1.5, 2, 3, 4, 6, and 24 h after BL-P or NB-P consumption. Blood samples were drawn into evacuated tubes with heparin as an anticoagulant. Urine was collected 24 h before the experiment and then 24 and 48 h after BL-P or NB-P intake. To check for compliance to the dietary instructions, a 1 day food record was kept by subjects 2 days before and 1 day after the intake of the blueberry purées. Moreover, a direct interview was scheduled with a dietitian.

Physicochemical and Phenolic Analyses of the IQF Berries and Blueberry Purées. Physicochemical and phenolic analyses were carried out in duplicate on IQF berries and frozen purée samples, after a thawing time of 4 h at room temperature. For berries, two aliquots of 300 g of IQF fruit were collected and homogenized after thawing; analyses were conducted in duplicate on homogenized portions. For each NB and BL purée samples, two vessels (2 × 300 g) were thawed and analysis was conducted in duplicate on the product. Total titratable acidity (TA), pH, and soluble solids content (SSC) were determined in duplicate according to the Official Methods of Analysis.[30] SSC was measured using a refractometer (RFM 81, Bellingham-Stanley, Ltd., Kent, U.K.), and TA was measured by titrating 5 g of each sample plus 50 mL of distilled water with 0.1 N NaOH to pH 8. Phenolic compounds were extracted in duplicate with a formic acid/water (5:95, v/v) solution according to Brambilla et al.[24] A subsample (15 g) of each blueberry product was homogenized with the extraction solution (25 mL) and left at room temperature for 20 min in the dark before centrifuging at 6000 rpm for 5 min (PK 130 Centrifuge, ALC International, Milan, Italy). The pellet following centrifugation was washed with 25 mL of the extraction solution and centrifuged at 6000 rpm for 5 min, and the resulting supernatant was combined with the initial supernatant.

Table 1. Physicochemical Parameters and Vitamin C Content of IQF Berries and Unblanched (NB-P) and Blanched (BL-P) Blueberry Purées[a]

	IQF berries	NB-P	BL-P
SSC (°Brix)	12.75 ± 0.04 a	11.54 ± 0.06 b	11.13 ± 0.03 c
pH	2.70 ± 0.05 a,b	2.63 ± 0.06 b	2.73 ± 0.05 a
titratable acidity (meq/100 g of FW)	17.20 ± 0.18 a	17.41 ± 0.51 a	15.41 ± 0.10 b
ascorbic acid (mg/100 g of FW)	0.80 ± 0.10 a	0.81 ± 0.11 a	0.60 ± 0.12 a

[a]Data are expressed as the mean ± standard deviation (SD). Data with different letters in the same row are statistically different at $p \leq 0.05$ (Tukey's test).

Spectrophotometric analysis of total phenolic content (TPC), monomeric ACN pigment (MAP), and polymeric color (PC, %) was carried out on the extracts with a UV4 ultraviolet–visible (UV–vis) spectrometer (Unicam, Cambridge, U.K.). TPC, expressed as gallic acid equivalents (GAE), was measured by the Folin–Ciocalteau assay. MAP, expressed as cyanidin 3-glucoside equivalents (C3GE), was measured by a pH-differential method. PC (%) was measured by a bisulfite bleaching method.[31,32] Chlorogenic acid and individual ACN compounds were analyzed by gradient-reversed-phase high-performance liquid chromatography (RP-HPLC) and diode array detection (DAD) according to Brambilla et al.[24] The chromatographic system consisted of a PU 1580 pump (Jasco Co., Tokyo, Japan), a 250 × 4.6 mm inner diameter, 5 μm, Inertsil ODS-3 column, heated at 40 °C, and a Jasco MD 2010 Plus photodiode array detector. The mobile phases consisted of acetonitrile and water/formic acid (90:10, v/v), and elution was performed at a flow rate of 0.5 mL/min by linear gradient steps. All of the ACN monoglycosides were expressed as C3GE, and chlorogenic acid was expressed as milligrams per 100 g of fresh weight (FW) by measuring detector response to the commercial standard.

Vitamin C (Ascorbic Acid) Analysis of the IQF Berries and Blueberry Purées. The ascorbic acid was extracted and determined by HPLC analysis according to Riso et al.[33] Briefly, a sample (5 g), in duplicate, of IQF berries and blueberry purées (BL-P and NB-P) was homogenized and suspended in 10 mL of MPA (10%), centrifuged at 3000g for 1 min, filtered, and injected for HPLC analysis. Ascorbic acid was determined by means of a chromatographic system consisting of a model 510 system pump (Waters), a 5 μm Atlantis C18 column (250 × 4.6 mm inner diameter, Waters, Ireland), and an UV–vis detector (Varian 9050). Samples were eluted with a mobile phase of 0.1% formic acid (1.4 mL/min), and the detection was achieved at 245 nm. The volume of injection was 50 μL. Chromatographic data were acquired by a Millenium 4.0 Workstation (Waters).

Plasma Separation. Plasma was separated within 30 min after collection by centrifugation for 15 min at 2300g at 4 °C. Two aliquots (1 mL) were acidified with TFA (1%) to preserve ACN stability and centrifuged at 4500g for 1 min, and the supernatants were stored at −80 °C for no longer than 2 months.

ACN Extraction and Analysis in Plasma. ACNs were extracted from plasma using a Micro-Plate solid-phase extraction (SPE) HLB Oasys Cartridge preactivated with methanol (500 μL) and washed with 500 μL acidified water (1% TFA). Plasma (400 μL) was diluted with 140 μL of acidified water (1% TFA) and 60 μL of water containing IS (50 ng/mL of CydG) used to correct the loss of ACNs during sample preparation. Samples were vortexed, centrifuged, and loaded onto the cartridge. Samples were drained under gravity, and the cartridge was washed with acidified water (100 μL; 1% TFA) and 100 μL of water/MeOH (80:20, v/v) acidified (0.1% TFA). The ACNs were eluted from the cartridge using 50 μL of methanol containing TFA (0.1%) to concentrate 8 times the amount of ACNs in plasma. The filtered sample was collected, and 20 μL was injected into an UHPLC MS/MS system for the analysis of individual ACNs, according to a method previously published.[34] The lower limit of detection was 1 ng/mL for Mv-glc, 4 ng/mL for D-glc, and about 2 ng/mL for the other ACNs.[34]

Hippuric Acid Extraction and Analysis in Urine. Urine (0.2 mL) was diluted with 1.8 mL of acidified water (0.1% formic acid) and centrifuged at 900g for 10 min. The supernatant was collected, and 20

μL was injected into the UHPLC system for the analysis according to a method previously published.[34]

Statistical Analysis. Statistical analysis on plasma samples was performed by means of STATISTICA software (Statsoft, Inc., Tulsa, OK), whereas that of the biochemical data on blueberry purées was carried out using the Statgraphics version 7 (Manugistic, Inc., Rockville, MD) software package. Data obtained on plasma levels of ACNs were analyzed by a two-way analysis of variation (ANOVA) for repeated measures design, using the type of product and time as dependent factors. Differences between means were further analyzed by the least significant difference (LSD) test. Differences were considered significant at $p \leq 0.05$. Data of physicochemical parameters of berries and blueberry purées, ACNs, and phenolic composition were analyzed by a one-way ANOVA procedure, and means were compared by Tukey's test ($p \leq 0.05$).

■ RESULTS AND DISCUSSION

The impact of food processing on ACN-rich products has not been thoroughly investigated. However, it is of crucial importance for the food industry; because it is recognized that thermal treatment is detrimental for nutrients and bioactive components of the food.[35] Thus, food processing based on mild technologies has been developed to preserve or enhance the nutritive value of foods. We investigated whether a blanching treatment could enhance ACN and phenolic content in a blueberry purée compared to a purée that did not undergo the blanching treatment. Table 1 reports the physicochemical parameters of IQF berries and NB-P and BL-P purées. Berries were characterized by a SSC of 12.75 °Brix and a titratable acidity of 17.2 meq/100 g of FW. In comparison to IQF berries, NB and BL purées exhibited a slight decrease in SSC, more pronounced in BL samples. BL-P also exhibited a decrease in titratable acidity compared to IQF berries and NB-P samples (−11% average value) and a corresponding increased pH value. The ascorbic acid content of IQF berries was relatively low (0.8 mg/100 g of FW) and did not significantly change following purée processing. The ACN and phenolic profiles of the purée are reported in Table 2. The TPC and chlorogenic acid content were higher in BL-P compared to NB-P, while PC (%), index of phenolic degradation products, was higher in NB-P. The total ACN content, computed either by the pH differential method or the RP-HPLC detection, was not significantly different between the BL and NB samples.

Galactoside and arabinoside forms were the prominent compounds detected in the blueberry products with Mv-3-gal and Dp-3-gal as the dominant ACNs, followed by Mv-3-ara, Cy-3-gal + Dp-3-ara, and Pt-3-glc (14.4, 13.3, and 10.6%, respectively) in the NB samples and Cy-3-gal + Dp-3, Mv-3-ara, and Pt-3-glc (16.7, 13, and 12.2%, respectively) in the BL samples. On the contrary, glucoside forms were detected at very low concentrations. Blanching had an influence on individual ACN amounts found in purées: the main differences concerning Dp-3-gal and Cy-3-gal + Dp-3-ara were higher in

Table 2. Composition of the Unblanched (NB-P) and Blanched (BL-P) Blueberry Purées[a]

	NB-P	BL-P
TPC (mg of GAE/100 g of FW)	242.43 ± 23.91 b	285.17 ± 11.17 a
MAP (mg of C3GE/100 g of FW)	88.78 ± 2.59 a	92.73 ± 2.84 a
PC (%)	7.52 ± 1.61 a	2.51 ± 0.47 b
chlorogenic acid (mg/100 g of FW)	30.09 ± 1.24 b	40.51 ± 3.21 a
total ACN (mg of C3GE/100 g of FW)	116.12 ± 6.98 a	119.48 ± 6.51 a
Dp-3-gal	19.04 ± 2.04 b	26.76 ± 2.83 a
Dp-3-glc	0.57 ± 0.11 a	0.41 ± 0.03 b
Cy-3-gal + Dp-3-ara	15.45 ± 1.27 b	19.37 ± 1.39 a
Cy-3-glc	0.51 ± 0.02 a	0.11 ± 0.05 b
Pt-3-gal	12.31 ± 1.44 a	14.18 ± 1.39 a
Cy-3-ara	1.77 ± 0.06 a	1.30 ± 0.10 b
Pt-3-glc	2.36 ± 0.10 a	2.18 ± 0.79 a
Peo-3-gal + Pt-3-ara	8.07 ± 0.31 a	8.60 ± 0.50 a
Peo-3-glc	1.26 ± 0.04 a	0.32 ± 0.08 b
Mv-3-gal	31.18 ± 1.54 a	26.85 ± 1.70 b
Mv-3-glc	2.72 ± 0.08 a	1.14 ± 0.19 b
Mv-3-ara	16.71 ± 0.82 a	15.07 ± 1.11 a

[a]TPC, total phenolic content; MAP, monomeric ACN pigment, PC, polymeric color; Dp-3-gal, delphinidin-3-galactoside; Dp-3-glc, delphidin-3-glucoside; Cy-3-gal + Dp-3-ara, cyanidin-3-galactoside + delphinidin-3-arabinoside; Cy-3-glc, cyanidin-3-glucoside; Pt-3-gal, petunidin-3-galactoside; Cy-3-ara, cyanidin-3-arabinoside; Pt-3-glc, petunidin-3-glucoside; Peo-3-gal + Pt-3-ara, peonidin-3-galactoside + petunidin-3-arabinoside; Peo-3-glc, peonidin-3-glucoside; Mv-3-gal, malvidin-3-galactoside; Mv-3-glc, malvidin-3-glucoside; and Mv-3-ara, malvidin-3-arabinoside. Data are expressed as the mean ± SD. Data with different letters in the same row are statistically different at $p \leq 0.05$ (Tukey's test).

BL-P (+40.5 and +25.3%, respectively), and Mv-3-gal was higher in NB-P (+16.1%). The content in ACN glucoside derivatives was relatively low in all of the samples but significantly higher in NB-P with respect to BL-P (7.42 versus 4.16 mg of C3GE/100 g of FW, respectively), especially for Dp-3-glc, Cy-3-glc, and Mv-3-glc.

These results are in accordance with data reported in the literature, in which a blanching step prior to purée and juice processing improved the retention or increased the content of phenols and ACNs in blueberries.[24,28,36]

The objective of the study was to investigate whether a blanching step for whole berries could affect the absorption of ACNs from the blueberry purées in healthy human volunteers. In this regard, we documented that ACN compounds were poorly absorbed following the consumption of a single portion (300 g) of BL-P and NB-P (providing about 358 mg of ACNs for BL-P and about 348 mg for NB-P). Only 3 of the 12 ACNs identified in the blueberry purées were detected in plasma. These compounds (Cy-3-glc, Mv-3-glc, and Dp-3-glc) were present at low concentrations in both purées. ACNs were rapidly absorbed, and their plasma concentration increased 1 h after consumption, achieving their maximum concentration at 1.5 h. These levels decreased after 2 and 3 h, reaching baseline values after 4 h (Figure 1). Two-way ANOVA did not show on the whole a significant effect of processing on ACN absorption but a significant effect of the time ($p \leq 0.0001$). However, post-hoc comparisons demonstrated that plasma ACN concentrations were different at specific time points: ACN absorption was higher (+25%; $p \leq 0.05$) after 1.5 h following BL-P intake

(31.1 ± 11.4 nmol/L) with respect to NB-P (24.9 ± 14.0 nmol/L). Moreover, the plasma ACN concentration tended to decrease after 2 h with respect to 1.5 h for both the purées but remained significantly higher up to 2 h following only the intake of BL-P (24.9 ± 10.1 versus 14.3 ± 9.6 nmol/L; $p \leq 0.001$) (Figure 1A).

Considering the absorption of single ACNs, we observed a similar trend for malvidin and cyanidin. In particular, we documented a significant ($p < 0.001$) increase of Mv-3-glc after the intake of BL-P and NB-P. The concentration remained significantly higher up to 2 h after consumption with respect to 1 h following the intake of BL-P (15.9 ± 7.5 versus 9.8 ± 7.3 nmol/L; $p \leq 0.01$). Mv-3-glc was the only ACN detected after 3 h from the intake of both purées (1.7 ± 1.7 nmol/L in BL-P and NB-P) (Figure 1B). The same trend was observed for Cy-3-glc, even if plasma concentrations were lower with respect to Mv-3-glc. Plasma Cy-3-glc concentrations were higher after BL-P intake with respect to NB-P at both 1.5 h (11.7 ± 4.3 nmol/L for BL-P and 9.5 ± 5.2 nmol/L for NB-P; $p \leq 0.05$) and 2 h (9.0 ± 3.2 nmol/L for BL-P and 6.2 ± 3.2 nmol/L for NB-P; $p \leq 0.05$) (Figure 1C). The ACN concentration remained significantly higher up to 2 h with respect to 1 h following the intake of BL-P (9.0 ± 3.2 versus 4.5 ± 2.6 nmol/L; $p \leq 0.01$). Additionally, Dp-3-glc exhibited similar time patterns as the other ACNs; however, this compound was detected at low concentrations only in the plasma of a few volunteers (4 of 10; data not shown) who were also those having higher absorption of Mv- and Cy-3-glc, suggesting subject-specific absorption.

From this study, three important conclusions can be drawn. The first concerns the confirmation of the low absorption of ACNs. In fact, the concentration of ACNs detected in the plasma of our subjects was comparable to that found by other authors after blueberry or elderberry intake.[11,37,38] However, our data differ from these studies, and it concerns the number of ACNs that were absorbed. We were able to detect only 3 ACNs in the plasma of our subjects compared to 12 found in our blueberry purées. This result is not surprising because it is widely recognized that the absorption of ACNs is also strictly dependent upon the food matrix (i.e., whole food, a drink, a juice, or a purée).[28] The second consideration is related to the absorption of the ACN glucosidic forms with respect to the others (galactoside or arabinoside), which could be due to a preferential uptake of glucose through a pathway involving the cotransporter SGLT-1.[28] Glucose transporters SGLT1 and GLUT2 have been reported to transport Cy-3-O-glc and Mv-3-O-glc.[39] Moreover, the higher absorption of Cy-3-glc present at low concentrations (~4-fold lower for NB-P and ~10-fold lower for BL-P compared to Mv-3-glc) could be attributed to the elevated hydrophilicity of this compound with respect to the other glucosidic forms.[40] The third consideration concerns the higher absorption of the ACNs from the BL-P, even though the ACN content in BL-P was not significantly different from that in NB-P. In this regard, the differential ACN absorption could have been influenced by an improved bioaccessibility of ACNs from the matrix of the blanched products; in fact, there is some evidence of an increased delocalization of pigments in blueberry tissues consequent to blanching.[26]

In a previous animal study, we documented that the urinary excretion of hippuric acid increased significantly after 4 and 8 weeks of administration of a wild blueberry-enriched diet.[34] Even though hippuric acid is the final product of the metabolic pathway of amino acids and fiber, its content in urine could represent a potential biomarker of ACN absorption and
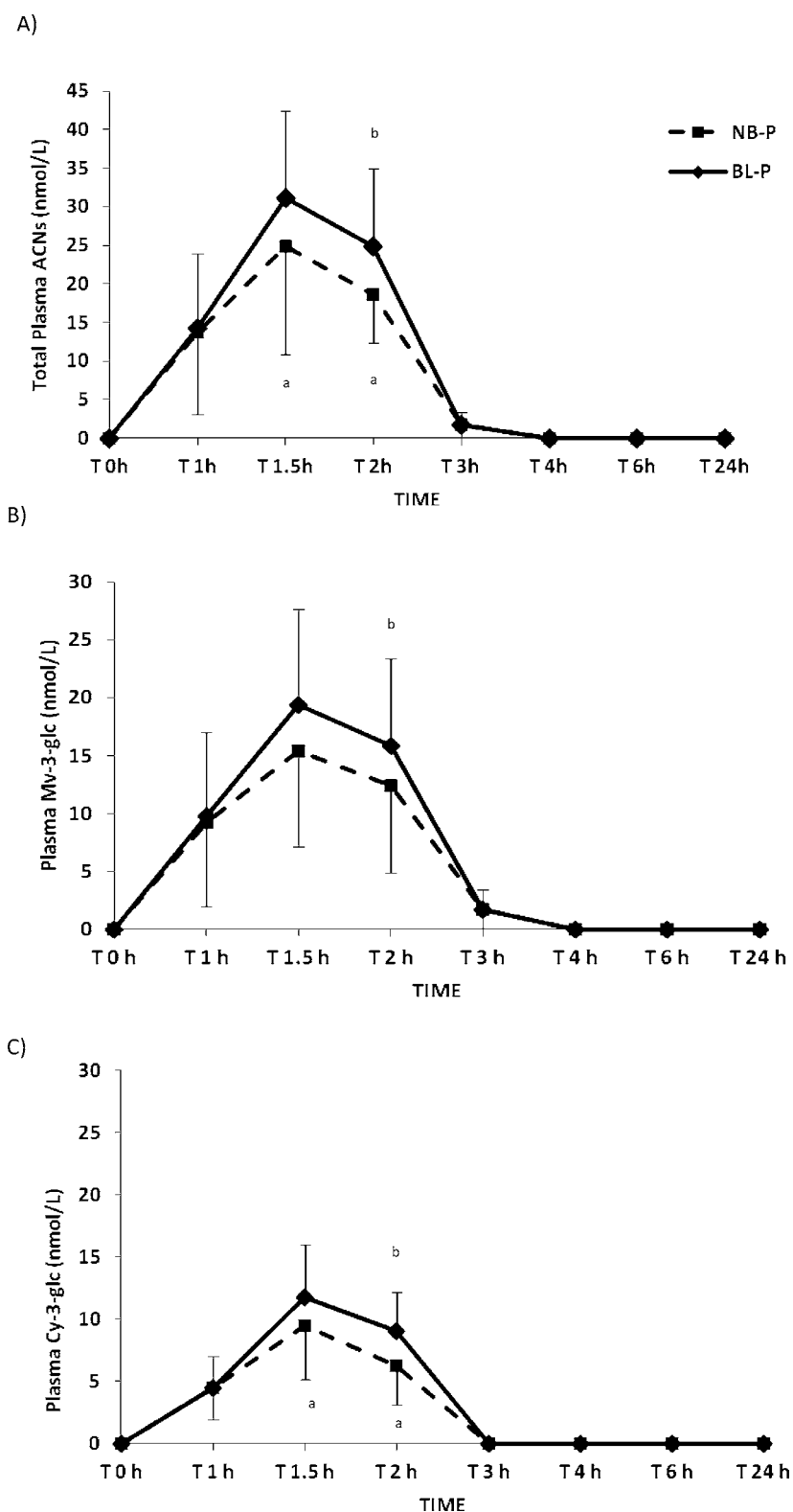
Figure 1. (A) Total plasma ACN, (B) Mv-3-glc, and (C) Cy-3-glc concentrations after the consumption of 300 g of unblanched (NB-P) and blanched (BL-P) blueberry purées. Data are present as the mean ± SD. (a) BL-P was significantly different with respect to NB-P at 1.5 and 2 h; $p \leq$ 0.05. (b) BL-P at 2 h was significantly different with respect to BL-P at 1 h; $p \leq$ 0.05.

metabolism and provide information on diet exposure.[41] In the present study, the levels of hippuric acid (expressed as total milligrams) detected in the urine collected 24 h before and 24 and 48 h after the consumption of BL-P and NB-P purée are reported in Figure 2. Statistical analysis did not show a

significant effect of the type of purée but confirmed a significant effect of time ($p < 0.05$). The intake of both purées significantly increased the excretion of hippuric acid after 24 h of blueberry consumption in both groups: BL-P (223 ± 124 versus 469 ± 282 mg total; $p < 0.05$) and NB-P (210 ± 101 versus 544 ±
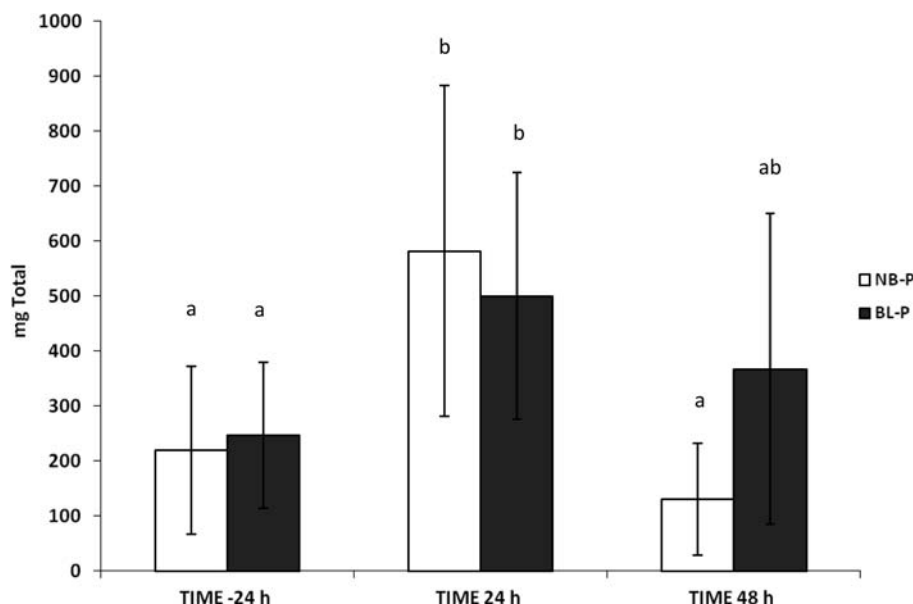
Figure 2. Total hippuric acid concentration in urine before and 24 and 48 h after the consumption of 300 g of unblanched (NB-P) and blanched (BL-P) blueberry purées. NB-P, unblanched blueberry purée; BL-P, blanched blueberry purée. Data are present as the mean ± SD. Data with different letters are significantly different at $p \leq 0.05$.

311 mg total; $p < 0.05$). The levels of hippuric acid decreased after 48 h from NB-P intake (130 ± 102 mg total), while they remained higher, even if not significantly, after the BL-P intake (367 ± 283 mg total) with respect to 24 h pre-intervention. Our results suggest that ACNs and other phenolic compounds were absorbed, metabolized, and excreted as hippuric acid within 24−48 h after the consumption of both purées.

In conclusion, with this pilot study, even though the mild blanching process applied had no significant effect on total ACN content possibly because of the low degree of exposure to native enzymes responsible for ACN degradation typical of the juice-making process, it is important to observe that berry blanching enhanced ACN absorption from minimally processed purées 1.5 and 2 h after consumption.

This may be especially important for both processors and researchers in this area. The influence of processing on ACN metabolism and health effects *in vivo* should be the focus of future research.

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Notes
The authors declare no competing financial interest.

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